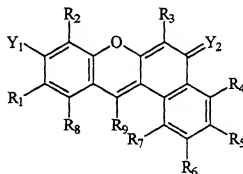


WE CLAIM:

1. An asymmetric benzoxanthene dye compound having the formula:

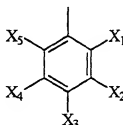


5 wherein:

Y_1 and Y_2 taken separately are selected from the group consisting of hydroxyl, oxygen, imminium, and amine;

R_1 - R_8 taken separately are selected from the group consisting of hydrogen, fluorine, chlorine, lower alkyl, lower alkene, lower alkyne, sulfonate, sulfone, amino, imminium, amido, nitrile, lower alkoxy, linking group, and combinations thereof; and

R_9 is selected from the group consisting of acetylene, lower alkyl, lower alkene, cyano, phenyl, substituted phenyl, heterocyclic aromatic, and combinations thereof, the substituted phenyl having the structure:



15 wherein:

X_1 - X_5 taken separately are hydrogen, chlorine, fluorine, lower alkyl, carboxylic acid, sulfonic acid, $-CH_2OH$, or linking group.

2. The compound of **claim 1** wherein one of Y₁ and Y₂ is oxygen and the other is hydroxyl.

3. The compound of **claim 1** wherein:

5 X₁ is selected from the group consisting of carboxylic acid, sulfonic acid, and -CH₂OH;

X₂ and X₃ taken separately are selected from the group consisting of hydrogen, chlorine, fluorine, and lower alkyl; and

10 X₃ and X₄ taken separately are selected from the group consisting of hydrogen, chlorine, fluorine, lower alkyl, carboxylic acid, sulfonic acid, and linking group.

4. The compound of **claim 1** wherein X₂ and X₃ are chlorine.

5. The compound of **claim 1** wherein X₁ is carboxylic acid.

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6. The compound of **claim 1** wherein one of X₃ or X₄ is linking group, the other being hydrogen.

7. The compound of **claim 1** wherein one of X₁ or X₅ is selected from the group
20 consisting of carboxylic acid, sulfonic acid, and -CH₂OH.

8. The compound of **claim 1** wherein one of R₁-R₃ is fluorine.

9. The compound of **claim 8** wherein R₃ is fluorine.

25

10. The compound of **claim 1** wherein:

one of Y₁ and Y₂ is oxygen and the other is hydroxyl;

R₁ is a chlorine;

R₃ is a fluorine;

R_2 and R_4 - R_8 are hydrogen; and
 R_9 is substituted phenyl wherein X_1 is carboxyl, X_2 and X_3 are chlorine, and one of X_3
 and X_4 is carboxyl and the other is hydrogen.

- 5 11. The compound of claim 1 wherein:
 one of Y_1 and Y_2 is oxygen and the other is hydroxyl;
 R_1 and R_3 are fluorine;
 R_2 , and R_4 - R_8 are hydrogen; and
 R_9 is substituted phenyl wherein X_1 is carboxyl, X_2 and X_3 are chlorine, and one of X_3
 10 and X_4 is carboxyl and the other is hydrogen.

12. The compound of claim 1 wherein:
 one of Y_1 and Y_2 is oxygen and the other is hydroxyl;
 R_1 is methoxy, R_2 is chlorine, R_3 is fluorine;
 15 R_4 - R_8 are hydrogen; and
 R_9 is substituted phenyl wherein X_1 is carboxyl, X_2 and X_3 are chlorine, and one of X_3
 and X_4 is carboxyl and the other is hydrogen.

13. The compound of claim 1 wherein:
 20 one of Y_1 and Y_2 is oxygen and the other is hydroxyl;
 R_3 is fluorine; R_1 , R_2 , and R_4 - R_8 are hydrogen; and
 R_9 is substituted phenyl wherein X_1 is carboxyl, X_2 and X_3 are chlorine, and one of X_3
 and X_4 is carboxyl and the other is hydrogen.

- 25 14. The compound of claim 1 wherein:
 one of Y_1 and Y_2 is oxygen and the other is hydroxyl;
 R_1 - R_8 are hydrogen; and
 R_9 is substituted phenyl wherein X_1 is carboxyl, X_2 and X_3 are chlorine, and one of X_3
 and X_4 is carboxyl and the other is hydrogen.

15. The compound of **claim 1** wherein:

one of Y_1 and Y_2 is oxygen and the other is hydroxyl;

R_1 is chlorine; R_2 - R_4 are hydrogen; and

5 R_9 is substituted phenyl wherein X_1 is carboxyl, X_2 and X_3 are chlorine, and one of X_3 and X_4 is carboxyl and the other is hydrogen.

16. The compound of **claim 1** wherein:

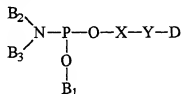
one of Y_1 and Y_2 is oxygen and the other is hydroxyl;

10 R_1 is methoxy; R_2 is chlorine;

R_3 - R_8 are hydrogen; and

R_9 is substituted phenyl wherein X_1 is carboxyl, X_2 and X_3 are chlorine, and one of X_3 and X_4 is carboxyl and the other is hydrogen.

15 17. A phosphoramidite compound having the formula:



wherein:

X is a spacer arm;

Y is a linkage;

20 B_1 is a phosphite ester protecting group;

B_2 , and B_3 taken separately are selected from the group consisting of lower alkyl, lower alkene, aryl, and cycloalkyl containing up to 10 carbon atoms; and

D is the dye compound of Claim 1;

wherein Y and D are linked through a linkage attached to dye D at one of positions

25 R_1 - R_9 .

18. The compound of **claim 17** wherein B_2 and B_3 taken together form an alkene chain containing up to 5 carbon atoms in the principle chain and a total of up to 10 carbon atoms with both terminal valence bonds of said chains being attached to the nitrogen atom; or B_2 and B_3 taken together with the nitrogen atom form a saturated nitrogen heterocycle which contains
 5 one or more heteroatoms selected from the group consisting of nitrogen, oxygen, and sulfur.

19. The compound of **claim 18** wherein:

B_1 is selected from the group consisting of methyl, β -cyanoethyl, or 4-nitrophenylethyl;

- 10 B_2 and B_3 taken separately are selected from the group consisting of isopropyl, t-butyl, isobutyl, and sec-butyl; and

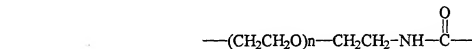
B_2 and B_3 taken together is morpholino.

20. The compound of **claim 17** wherein X and Y taken together is



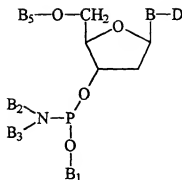
wherein n ranges from 2 to 10.

21. The compound of **claim 17** wherein X and Y taken together is



wherein n ranges from 2 to 10.

22. A phosphoramidite compound having the formula:



wherein:

B₁ is a phosphite ester protecting group;

- B₂, and B₃ taken separately are selected from the group consisting of lower alkyl,
 5 lower alkene, aryl, and cycloalkyl containing up to 10 carbon atoms;

B₃ is an acid-cleavable hydroxyl protecting group;

B is a nucleotide base; and

D is the dye compound of Claim 1;

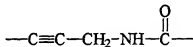
- wherein when B is purine or 7-deazapurine, the sugar moiety is attached at the N⁹-
 10 position of the purine or 7-deazapurine, and when B is pyrimidine, the sugar moiety is attached
 at the N¹-position of the pyrimidine;

wherein B and D are linked through a linkage attached to D at one of positions R₁-R₉;

and

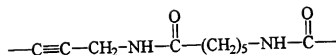
- wherein if B is a purine, the linkage is attached to the 8-position of the purine, if B is 7-
 15 deazapurine, the linkage is attached to the 7-position of the 7-deazapurine, and if B is
 pyrimidine, the linkage is attached to the 5-position of the pyrimidine.

23. The compound of claim 22 wherein the linkage is

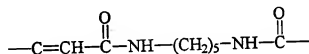


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24. The compound of claim 22 wherein the linkage is



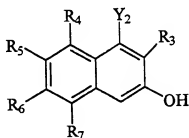
25. The compound of **claim 22** wherein the linkage is



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26. The compound of **claim 22** wherein B is selected from the group consisting of uracil, cytosine, deazaadenine, and deazaguanosine.

27. A compound having the formula:



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wherein:

R_3 - R_7 taken separately are selected from the group consisting of hydrogen, fluorine, chlorine, lower alkyl, lower alkene, lower alkyne, sulfonate, amino, amido, nitrile, lower alkoxy, and linking group; and

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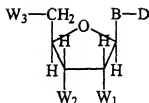
Y_2 is selected from the group consisting of hydroxyl and amine.

28. The compound of **claim 27** wherein R_3 is fluorine.

29. The compound of **claim 27** wherein Y_2 is hydroxyl.

20

30. A labeled nucleotide having the formula:

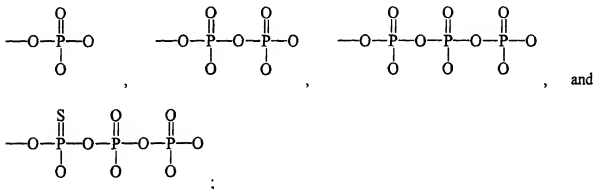


wherein:

B is a 7-deazapurine, purine, or pyrimidine nucleotide base;

W_1 and W_2 taken separately are selected from the group consisting of H and OH;

5 W_3 is selected from the group consisting of OH,



D is the dye compound of Claim 1;

10 wherein when B is purine or 7-deazapurine, the sugar moiety is attached at the N⁹-position of the purine or deazapurine, and when B is pyrimidine, the sugar moiety is attached at the N¹-position of the pyrimidine;

wherein the linkage linking B and D is attached to D at one of positions R₁-R₉; and

15 wherein if B is a purine, the linkage is attached to the 8-position of the purine, if B is 7-deazapurine, the linkage is attached to the 7-position of the 7-deazapurine, and if B is pyrimidine, the linkage is attached to the 5-position of the pyrimidine.

31. The labeled nucleotide of **claim 30** wherein B is selected from the group consisting of uracil, cytosine, deazaadenine, and deazaguanosine.

20 32. The labeled nucleotide of **claim 30** wherein the linkage is

and, D is a dye compound of Claim 1;

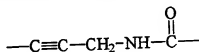
wherein when B is purine or 7-deazapurine, the sugar moiety is attached at the N⁹-position of the purine or deazapurine, and when B is pyrimidine, the sugar moiety is attached at the N¹-position of the pyrimidine;

5 wherein the linkage linking B and D is attached to D at one of positions R₁-R₉; and

wherein if B is a purine, the linkage is attached to the 8-position of the purine, if B is 7-deazapurine, the linkage is attached to the 7-position of the 7-deazapurine, and if B is pyrimidine, the linkage is attached to the 5-position of the pyrimidine.

10 36. The labeled polynucleotide of claim 35 wherein B is selected from the group consisting of uracil, cytosine, deazaadenine, and deazaguanosine.

37. The labeled polynucleotide of claim 35 wherein the linkage is



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38. A method of polynucleotide sequencing comprising the steps of:

forming a mixture of a first, a second, a third, and a forth class of polynucleotides such

that:

20 each polynucleotide in the first class includes a 3'-terminal dideoxyadenosine and is labeled with a first dye;

each polynucleotide in the second class includes a 3'-terminal dideoxycytidine and is labeled with a second dye;

each polynucleotide in the third class includes a 3'-terminal dideoxyguanosine and is labeled with a third dye; and

25 each polynucleotide in the forth class includes a 3'-terminal dideoxythymidine and is labeled with a forth dye;

wherein one of the first, second, third, or forth dyes is the asymmetric benzoxanthene dye of Claim 1;

the other of the dyes being spectrally resolvable from the asymmetric benzoxanthene dye and from each other;

5 electrophoretically separating the polynucleotides thereby forming bands of similarly sized polynucleotides;

illuminating the bands with an illumination beam capable of causing the dyes to fluoresce; and

10 identifying the classes of the polynucleotides in the bands by the fluorescence spectrum of the dyes.

39. The method of **claim 38** wherein the other of the dyes are selected from the group consisting of 6-carboxyfluorescein, 6-carboxy-4,7,2',7'-tetrachlorofluorescein, and 6-carboxy-4,7,2',4',5',7'-hexachlorofluorescein.

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40. A method of fragment analysis comprising:

forming a labeled polynucleotide fragment, the fragment being labeled with the dye compound of Claim 1;

20 subjecting the labeled polynucleotide fragment to a size-dependent separation process; and

detecting the labeled polynucleotide fragment subsequent to the separation process.

41. The method of **claim 40** wherein the size-dependent separation process is electrophoresis and the detecting is by fluorescence.